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(54) Title: A METAL PARTICLE, ITS PREPARATION AND USE, AND A MATERIAL OR DEVICE COMPRISING THE METAL PARTICLE

(57) Abstract

A metal particle comprising a metal core and a silane shell, wherein said core comprises a metal selected from the group consisting of gold, silver, platinum, palladium, rhodium, ruthenium, osmium, iridium and combinations thereof, and said shell comprises a mercaptosilane residue bound to said metal core. The silane shell may be surrounded by a further shell, which comprises a silicate, titanate, zirconate, aluminate, borate or, preferably, silane residue. The residues in this further shell may be cross–linked with each other. The particle may be a colloidal particle, and its size may be 5 nm or lower, such as 0.8 to 1.5 nm. The particle may carry a covalently attached foreign molecule. A method for preparing the particle, a material or device comprising the particle embedded in a matrix or carried on a support, and various uses of the particle.

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Title: A metal particle, its preparation and use, and a material or device comprising the metal particle

FIELD OF THE INVENTION

This invention is in various technical fields, including chemistry involved in designing and making metal particles, in particular colloidal metal particles comprising Au, Pt, Pd and/or Ag having a size in the order of a few nanometers or less, and various fields in which such metal particles may be used, such as in nanoelectronic devices and methods involving the use of metal quantum dots, (photo)catalytic materials and methods, kits and methods for detecting or assaying (e.g. for diagnostic purposes) immunological substances or nucleic acids involving the use of a colloidal metal particle-labeled reagent, and the art of ceramic monoliths and coatings.

More particularly, this invention relates to a metal particle, a material or device comprising same, a method for preparing said metal particle and use of said metal particle, wherein the metal is selected from the group consisting of gold, silver, platinum, palladium, rhodium, ruthenium, osmium and iridium.

BACKGROUND OF THE INVENTION

Metal nanoparticle preparation

Generally, colloidal metal sol preparation comprises the reduction of a molecular metal species (ionic metal salt or organometallic complex) in solution. The stabilization of the metal particles is brought about by ligand molecules attached to their surface. Effective ligand molecules are those that form strong bonds with the metal surface. Especially suitable are those ligands containing triphenyl phosphine or thiol groups which show a strong chemisorption on metal surfaces. Also adsorbing on metal, but to a lesser extent, are ligands containing amine or ammonium groups. Using these types of ligands, extremely small metal clusters can be formed which contain only a few tens of metal atoms.

Examples of triphenyl-phosphine-stabilized gold clusters are the $Au_{55}[P(phenyl)_3]_{12}Cl_6$ two-shell cluster having a core diameter of about 1.4 nm (*G. Schmid (1992) Chem. Rev. 92: 1709*) and the undecagold cluster $Au_{11}[P(aryl)_3]_7(CN)_3$ which has a core diameter of 0.8 nm (*P.A. Bartlett, B. Bauer, and S.J. Singer (1978) J. Am. Chem. Soc. 100: 5085*). Examples of phosphine-stabilized bimetallic clusters are $Au_{13}Ag_{12}[P(p\text{-}tolyl)_3]_{10}Cl_8^+$, $Ni_{34}Se_{22}[P(phenyl)_3]_{10}$, $Cu_{36}Se_{18}[P(t\text{-}Bu)_3]_{12}$, $Pd_{20}As_{12}P(phenyl)_3]_{12}$, and $Pd_9Sb_6[P(phenyl)_3]_8$ (*G. Schmid (1992) Chem. Rev. 92:1709*).

An example of alkylammonium-stabilized metal clusters are the mono- and bimetallic 2 to 5 nm clusters of Pt, Pd, Rh, Ru, Os, Mo, $Pt_{50}Sn_{50}$, $Cu_{44}Pd_{56}$, and $Pd_{50}Pt_{50}$ that have been (electrochemically) prepared by stabilizing with tetraalkyl-ammonium salt (a surfactant molecule) (*M.T. Reetz and S.A. Quaiser (1995) Angew. Chem. Int. Ed. Engl. 34: 2240*). Another type of stabilizer is phenanthroline, a nitrogen-containing fused aromatic ring compound with sulfonate groups. Examples for platinum and palladium are the phenanthroline-stabilized four-shell Pt_{309} cluster (*G. Schmid, B. Morun, J.-O. Malm (1989) Angew. Chem. 101: 772*) and the 3.6 nm phenanthroline-stabilized Pd cluster (*G. Schmid (1991) Mater. Chem. Phys. 29: 133*).

For the case of the thiols, Brust et al. (*M. Brust, M. Walker, D. Bethell, D.J. Schiffrin, and R. Whyman (1994) J.Chem.Soc., Chem.Commun. p. 80*) demonstrated that one can employ the ability of alkanethiols to form self-assembled monolayers (*R.G. Nuzzo, L.H. Dubois, and D.L. Allara (1990) J. Am. Chem. Soc. 112: 558; P.E. Laibinis, G.M. Whitesides, D.L. Allara, Y.-T Tao, A.N. Parikh, and R.G. Nuzzo (1991) J.Am.Chem.Soc. 113: 7152*) on gold for the preparation of gold nanoparticles. In their method, $AuCl_4^-$ was reduced in the presence of dodecanethiol to produce a stable colloid of thiol-capped gold nanoparticles of decreasing diameter with decreasing $AuCl_4^-$-to-thiol ratio. The role of the alkanethiol can be regarded as analogous to the one of the surfactant in

stabilizing water-in-oil microemulsions. By using the bifunctional stabilizer p-mercaptophenol, gold particles have been provided with functional phenolic hydroxyl groups (*M. Brust, J. Fink, D. Bethell, D.J. Schiffrin, and C. Kiely (1995), J. Chem. Soc., Chem. Commun. p. 1655*). Not only gold, but also platinum and palladium nanoparticles have been stablized by dodecanethiol ligands (*K.V. Sarathy, G.U. Kulkarni, C.N.R. Rao (1997) Chem. Commun. 1997, p.537*).

Metal quantum dots

When the diameter of metal particles is reduced to being in the quantum-size regime (smaller than about 2 nm), these particles show strong deviations from bulk metal behavior concerning their electric susceptibility and specific heat. These particles are called quantum dots, based on the view of the conduction-band electrons as particles in a box. The conduction electrons in small metal particles form standing electron waves with multiples of half the De Broglie wave-length ($\lambda/2$). When the metal particle is reduced to about a few dozens to hundreds of atoms, their size becomes of the order of $\lambda/2$ and the quasi-continuous density of states turns into a discrete energy level structure (quantum size effect; QSE). An effect that results from the QSE is the single-electron tunneling (SET), which may be of great importance for the ultimate miniaturization in nano-electronics. Two quantum-sized metal particles separated by an insulating layer of about 2 nm thickness or smaller can be regarded as a tunnel junction, where single electrons can transfer due to Coulomb interaction generated by an external bias voltage.

Especially appropriate for SET applications are the ligand-stabilized metal clusters, e.g. the $Au_{55}(PPh_3)_{12}Cl_6$ cluster with gold core diameter 1.4 nm and total diameter 2.1 nm (*G. Schmid, R. Pfeil, R. Boese, F. Bandermann, S. Mayer, G.H.M. Calis, J.W.A. van der Velden (1981) Chem. Ber. 114: 3634; Schmid (1984) US Patent no. 4,454,070*). The organic ligand shell stabilizes the metal core and prevents

coalescence of the cores, thus preserving their identity as quantum dots. The cores consist of geometric magic numbers of metal atoms, resulting from subsequently building up an n^{th} shell of $10n^2+2$ atoms around the central atom (densest sphere packing). The amounts of atoms in the particles are: 13, 55, 147, etc. This way very narrow size distributions of clusters are obtained.

The electron-hopping behavior (tunnel resistance and capacitance) of the particles depends on (1) the size and monodispersity of the metal core, (2) the thickness of the ligand shell, and (3) the chemical nature of the ligand. Especially important is the particle monodispersity. In a polydisperse particle assembly, the precise electronic energy level structure will vary from particle to particle, resulting after assembly averaging in a distribution of energy levels. For application in nano-structured materials, it is essential for a controlled single-electron-hopping conductivity that the gold nanoparticles are kept apart at a constant distance. Electron-hopping conductivities were measured for gold particles stabilized by a ligand shell of alkanethiolate molecules by Terrill et al. (*R.H. Terrill, T.A. Postlethwaite, C.-H. Chen, C.-D. Poon, A. Terzis, A. Chen, J.E. Hutchison, M.R. Clark, G. Wignall, J.D. Londono, R. Superfine, M. Falvo, C.S. Johnson Jr., E.T. Samulski, and R.W. Murray (1995) J. Am. Chem. Soc. 117: 12537*).

Bioreagent-bound gold probes

Colloidal gold particles coupled to immunoreagents are used for detecting proteins, lipids, RNA and DNA in cell-biological electron-microscopy (EM) studies (*Colloidal Gold: Principles, Methods and Applications, Hayat, M.A. Ed.; Academic Press: San Diego, CA, 1989, Vols. 1, 2 and 3*). Faulk and Taylor (*W.P. Faulk and G.M. Taylor (1971) Immunochem. 8: 1081*) were the first in preparing an immunoglobulin-colloidal gold complex, based on adsorption interaction, for cyto-chemistry at the transmission electron microscope (TEM)

level. The method was further developed by Horisberger et al. (*M. Horisberger and J. Rosset (1977) J. Histochem. Cytochem. 25: 295*). Since then, a number of proteins and glycoproteins such as immunoglobulins, lectins, and enzymes have been adsorbed on colloidal gold and applied without a reduction in their biological activities.

The adsorption of macromolecules onto the gold surface is a complex phenomenon, depending on the stability of the colloid, concentration, shape, configuration and iso-electric point of the macromolecules, and ionic strength, pH and temperature of the suspending medium. The monolayer of adsorbed macromolecules stabilizes the hydrophobic colloidal gold particles in aqueous solvent by electrosteric stabilization. However, usually colloidal gold conjugation requires an extra stabilizer (e.g. bovine serum albumin) to minimize aggregation. With increasing protein concentration, an increasing amount of protein is adsorbed on the gold particles as loosely bound multilayers, making the conjugate quite large in size. Colloidal gold-antibody probes based on adsorption mostly suffer a certain degree of dissociation of the colloidal gold, leading to its aggregation. The free IgG will then compete for labeling sites and reduce the number of antigens visualized by EM.

A covalent binding of an immunoreagent to colloidal gold will result, after purification, in a product without any free gold or free protein. Such a product will not show, as is the case for non-covalent gold-protein conjugates, a dissociation of gold due to the resetting of the adsorption equilibrium. Because of the lack of free gold particles, the labeling will show an improved reliability and a lower background. Because of the lack of free protein, the labeling will show an improved efficiency.

Colloidal gold conjugates (having gold particle diameter of ~ 5-50 nm) are too large to be efficiently applied for high-resolution EM studies and for studies demanding a high rate of penetration into cell structures. Ultrasmall gold

probes, smaller than about 1.4 nm (ofter called gold *clusters*) penetrate more easily into cell structures (e.g. the cell nucleus) and reach antigen binding sites better. Therefore, they may approach the labeling efficiency of fluorescently labeled antibodies. In order to visualize the ultra-small gold labels in the light microscope or in the standard electron microscope, silver enhancement of the particles is necessary. For the ultrasmall gold clusters, the on-growth of silver depends strongly on the cluster size. Therefore, to accomplish a homogeneous silver enhancement, it is important that the gold clusters have high monodispersity.

Hainfeld and Furuya developed an ultrasmall gold label with a gold core diameter of 1.4 nm and a shell composed of organophosphines bringing the overall diameter to 2.7 nm (*J.F. Hainfeld, and F.R. Furuya (1992) J. Histochem. Cytochem. 40: 177*). To the shell molecules, functional groups (e.g. maleimide) are attached that can serve for covalent linking to proteins. The 1.4 nm core most probably represents a two-shell gold cluster consisting of 55 gold atoms, comparable to the $Au_{55}[P(phenyl)_3]_{12}Cl_6$ cluster prepared by Schmid (*G. Schmid (1992)Chem. Rev. 92: 1709*). The smallest gold label yet developed for covalent conjugation is the undecagold cluster $Au_{11}[P(aryl)_3]_7(CN)_3$ with a gold core diameter of 0.8 nm and a total diameter of ~2 nm (*P.A. Bartlett, B. Bauer, and S.J. Singer (1978) J. Am. Chem. soc. 100: 5085; J.S. Wall, J.F. Hainfeld, P.A. Bartlett, and S.J. Singer (1982) Ultramicroscopy 8: 397; J.F. Hainfeld (1987) Science 236: 450*).

SUMMARY OF THE INVENTION

This invention provides a metal particle comprising a metal core and a silane shell, wherein said core comprises a metal selected from the group consisting of gold, silver, platinum, palladium, rhodium, ruthenium, osmium, iridium and combinations thereof, and said shell comprises a mercapto-silane residue bound to said metal core.

Preferably, said mercaptosilane residue is derived from a mercaptosilane compound of formula $HS-(CH_2)_m-Si(OR^1)_nR^2_{3-n}$, wherein m and n both are integers, $m \geq 0$, $0 \leq n \leq 3$, each R^1 independent from any further R^1's is a member of the group consisting of hydrogen, alkyl and trialkylsilyl, and each R^2 independent from any further R^2's is a member of the group consisting of alkyl, haloalkyl, phenyl and halogen. Most preferably,

--- $0 \leq m \leq 18$, preferably $1 \leq m \leq 6$, most preferably $m = 3$,

--- $0 \leq n \leq 3$, preferably $1 \leq n \leq 3$,

--- R^1 is hydrogen, C_1-C_6 alkyl, preferably C_1-C_4 alkyl, most preferably methyl or ethyl, or C_1-C_4 trialkylsilyl, most preferably trimethylsilyl, and

--- R^2 is C_1-C_{18} alkyl, preferably C_1-C_6 alkyl, halo(C_1-C_{18})-alkyl, preferably halo(C_1-C_6)alkyl, phenyl, or halogen, wherein halo(gen) is selected from the group consisting of F, Cl, Br and I.

The metal particle will usually have a size of 5 nm or lower, preferably a size in the range of from about 0.8 nm to about 1.5 nm. References to sizes concern (number) averages. Usually, at least 70% of the particles will satisfy the size limitations, preferably at least 80%, more preferably, at least 90%.

It is preferred that the mercaptosilane residues in said shell are cross-linked with each other.

In a further embodiment, said silane shell is surrounded by a further shell, said further shell comprising a silane residue, a silicate residue, a titanate residue, a zirconate residue, an aluminate residue, or a borate residue.

Preferably, said further shell comprises a silane residue derived from an organosilane compound of the formula $X-(CH_2)_m-Si(OR^1)_nR^2_{3-n}$, wherein m and n both are integers, $m \geq 0$, $0 \leq n \leq 3$, each R^1 independent from any further R^1's is a member of the group consisting of hydrogen, alkyl and trialkylsilyl, each R^2 independent from any further R^2's is a

member of the group consisting of alkyl, haloalkyl, fenyl and halogen, and X is a functional group. Most preferably,

--- X is selected from the group consisting of amino, thiol, carboxyl, aldehyde, dimethyl acetal, diethyl acetal, epoxy, cyano, isocyanate, acyl azide, anhydride, diazonium salt, sulfonate, hydroxyphenyl, aminophenoxy, halogen, acetate, acrylate, methacrylate and vinyl.

It is preferred that residues in said further shell are cross-linked with each other.

The metal particle of this invention preferably is a colloidal particle (i.e. capable of forming a stable sol or colloidal solution).

In a further embodiment, the metal particle carries a foreign molecule covalently attached to a silane residue, preferably to a silane residue in said further shell. Said foreign molecule is preferably selected from the group consisting of antibodies (immunoglobulines), antigens, haptens, biotin, avidin, streptavidin, protein A, proteins, enzymes, lectins, hormones, nucleic acids (DNA, RNA, oligo-nucleotides), fluorescent compounds (including fluoresceins and fluorochromes) and dyes.

This invention also provides a material or a device comprising a matrix or support having embedded therein or carrying a particle as defined herein, wherein said matrix or support is preferably selected from the group consisting of polymers, porous glass, colored glass, porous TiO_2, zeolites, silica, alumina, intercalated clay compositions, active carbon, graphite, ion-exchange resins, and semi-conductors such as p-GaAs, TiO_2, ZnO, CdS and Cd_3P_2.

Furthermore, this invention provides a method of making a metal particle comprising a metal core and a silane shell, wherein said core comprises a metal selected from the group consisting of gold, silver, platinum, palladium, rhodium, ruthenium, osmium, iridium and combinations thereof, and said shell comprises a mercaptosilane residue bound to said metal core, comprising treating a solution of a compound of the

metal with a reducing agent in the presence of a mercapto-silane compound.

In this method, the metal compound may be selected from salts of the metal, preferably soluble salts, in particular chloride, nitrate or nitrite salts, such as a salt from the group consisting of $HAuCl_4$, H_2PtCl_6, $PdCl_2$, $AgNO_3$, and similar Au, Pt, Pd and Ag salts. It is also possible to use two or more of such compounds together. Au, Pt, Pd and Ag are the preferred metals.

It is preferred that in this method the metal compound is dissolved in a polar solvent, preferably a polar solvent having a dielectric constant of 15 or higher, most preferably methanol, ethanol, dimethylsulfoxide, or dimethylformamide. It is also preferred that the reaction is carried out in the presence of water, preferably from about 1 to about 10% by weight (based on total weight of water and organic solvent).

Said reducing agent may, e.g., be a borohydride, such as sodium borohydride.

Preferably, the reducing agent and the metal compound are used in a molar ratio of 7 or higher, and preferably the metal compound and the mercaptosilane compound are used in a molar ratio of 6 or lower.

Furthermore, this invention provides a method wherein the metal particle comprising said silane shell is subjected to a further reaction, to surround said silane shell by a further shell, said further shell comprising a silane residue, a silicate residue, a titanate residue, a zirconate residue, an aluminate residue, or a borate residue.

Again, it is preferred that the reaction is carried out in a polar solvent, preferably a polar solvent having a dielectric constant of 15 or higher, most preferably methanol, ethanol, DMSO or DMF, in the presence of water, preferably from about 1 to about 10% by wt. (This weight percentage being based on the total weight of water and organic solvent.)

Furthermore, it is preferred that the reaction is carried out in the presence of a catalyst for siloxane bond formation, such as an amine or ammonia.

This invention also provides a method which in addition comprises a reaction to covalently attach a foreign molecule to a silane residue, preferably a silane residue in said further shell.

This invention also provides various uses of a metal particle as defined herein, in particular in nanoelectronic devices and methods, in (photo)catalytic materials and methods, in monoliths and coatings, as a label or labeled reagent in immunoassay kits and immunoassay methods, or as a label or labeled probe in nucleic acid detection kits and methods.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows a schematic illustration of the reaction sequence for the preparation of the mercaptosilane-stabilized metal particles and the coating of these with a secondary silane.

(a) The example of the reduction of tetrachloroaurate in the presence of γ-mercaptopropyltrimethoxysilane (MPS) to form MPS-stabilized gold particles in ethanol.

(b) The coating of the particles of (a) with γ-aminopropyl-triethoxysilane (APS) using ammonia catalyst. A siloxane cage is formed around the gold core.

FIGURE 2 shows electron micrographs of γ-mercaptopropyl-trimethoxysilane-stabilized gold particles.

(a) Transmission Electron Microscope (TEM) image of colloidal gold particles of about 3 to 5 nm prepared at a gold-to-mercaptosilane molar ratio of 5.29.

(b) TEM image of about 1.5 nm gold clusters prepared at a gold-to-mercaptosilane molar ratio of 0.529.

(c) High-angle annular-dark-field Scanning Transmission Electron Microscope (HAADF-STEM) image of the gold clusters of (b).

DETAILED DESCRIPTION OF THE INVENTION

The invention relates to the preparation and application of silane-stabilized metal particles of gold, platinum, palladium, silver, rhodium, ruthenium, osmium, iridium, or combinations thereof, which range in size from 5 nm colloids down to subnanometer clusters. The particles can be easily prepared at room temperature by the chemical reduction of an ionic metal species, e.g. Au, Pt, Pd or Ag species (e.g. $HAuCl_4$, H_2PtCl_6), preferably in a polar solvent of dielectric constant in excess of 15, such as in particular ethanol, methanol, dimethylsulfoxide (DMSO) and dimethylformamide (DMF), in the presence of a mercaptosilane compound like:

$$HS - (CH_2)_m - Si\ (OR^1)_n\ R^2_{3-n}$$

wherein

R^1 = H, alkyl, or trialkylsilyl;

R^2 = alkyl, halogen, haloalkyl, or phenyl;

m = a positive integer including zero;

n = 0, 1, 2 or 3.

Herein, when n = 2 or 3, each R^1 may be the same or different, and when n = 0 or 1, each R^2 may be the same or different. When R^1 is alkyl, it may be straight chain or branched chain alkyl. Preferably, each alkyl group is a lower alkyl, which may contain from 1 up to 6, preferably from 1 to 4, most preferably 1 or 2 carbon atoms. When R^1 is trialkylsilyl, each alkyl preferably is a lower alkyl, such as C_1-C_4 alkyl. Most preferably, when R^1 represents trialkylsilyl, it is trimethylsilyl. When R^2 is alkyl or haloalkyl, each alkyl group may be straight chain or branched chain alkyl and may contain from 1 up to 18 or more carbon atoms, preferably 1 to 6 carbon atoms, most preferably 1 to 4 carbon atoms. Halogen comprises F, Cl, Br and I. Haloalkyl may be fluoroalkyl, chloroalkyl, bromoalkyl and iodoalkyl. When R^2 is different from halogen, n may still be 0, 1, 2 or 3, but preferably is 1, 2 or 3, most preferably n = 3. The presence of at least

one alkoxy substituent facilitates subsequent binding of further chemicals. When R^2 is halogen, this halogen atom facilitates further reactions. The integer m may be 0 (zero), up to about 18 or higher. Preferably, m will be from 1 up to 6, and most preferably m is about 3.

As indicated above, it is preferred that the reaction is carried out in a polar solvent. By using a sufficiently polar solvent, the formation of colloidally stable mercaptosilane-stabilized metal particles is facilitated. When a solvent of too low polarity is used (say with dielectric constant below 12), the metal particles will form, but they will not retain their colloidal stability and will form flocs. This may be due to charge and/or solvation effects of the silane surface groups. For certain applications, however, it may not be detrimental or even be desirable that the particles lack colloidal stability.

The thiol group of the mercaptosilane has a high affinity for the metal, leading to a stabilizing chemisorbed mercaptosilane monolayer on the metal particle surface. The metal particle size is determined by the amount of metal surface formed by the self-assembling process of the mercaptosilane molecules on that surface at a certain metal-to-mercaptosilane ratio during the preparation. This way, the particle size can be controlled by the metal/mercaptosilane ratio; the smaller this ratio, the smaller the particles. The mercaptosilane stabilizes the metal nuclei in a manner which resembles the role of the surfactant in the spontaneous formation of a water-in-oil microemulsion. This should be distinguished from the situation in which metal colloids first homogeneously nucleate (and grow) and only afterwards are covered by stabilizing mercaptosilane molecules. The uniformity of the mercaptosilane particles increases with decreasing particle size. The metal clusters in the 0.8 to 1.5 nm size are highly monodispersed (Figure 2).

The mercaptosilane compound not only ensures colloidal stability of the metal particles, but also provides them,

when n is 1, 2 or 3, with alkoxysilane surface groups. The importance of this monolayer is its potentiality to react with the wide variety of (commercially available) organosilanes (*E.P. Plueddemann, Silane Coupling Agents; Plenum: New York, 1982*), to produce hybrid colloids with various properties. The (hydrolyzable) alkoxysilane groups of the mercaptosilane point outward from the metal particle and easily react via siloxane-bond-forming condensation reaction with a secondary silane compound which contains a functional group, like:

$$X - (CH_2)_m - Si\ (OR^1)_n\ R^2_{3-n}$$

wherein

R^1 = H, alkyl, or trialkylsilyl;

R^2 = alkyl, halogen, haloalkyl, or phenyl;

X = a functional group, e.g. amino, thiol, carboxyl, aldehyde, dimethyl acetal, diethyl acetal, epoxy, cyano, isocyanate, acyl azide, anhydride, diazonium salt, sulfonate, hydroxyphenyl, aminophenoxy, halogen, acetate, acrylate, methacrylate, vinyl;

m = a positive integer including zero;

n = 0, 1, 2 or 3.

Herein, the possible meanings of R^1, R^2, m and n are the same as for the mercaptosilane compound. The actual meaning of R^1, R^2, m and n in this secondary silane compound may be the same as, or different from the actual meaning of the same symbols in the mercaptosilane compound used.

By means of the silane cross-linking reaction of the secondary silane with the mercaptosilane on the metal-cluster surface, a cage-like siloxane-network structure is formed which wraps up the metal core (see Figure 1, illustrating monolayer coverage of both γ-mercaptopropyltrimethoxysilane and γ-aminopropyltriethoxysilane). This cross-linked-silane coat prevents the mercaptosilane molecules from being liberated from the metal core by for example high temperature

or a strongly reducing (thio)reagent applied during processing of the metal particles. The functional groups of the secondary silane molecules at the particle periphery can serve as the substrate for further covalent reaction with foreign molecules.

According to a first embodiment of the invention, first the secondary silane is coupled to the silane part of the mercaptosilane on the metal particle surface, after which a foreign molecule is bound to the functional group of the secondary silane.

In another embodiment of the invention, the coupling of foreign molecules onto the metal particles can be accomplished indirectly by first binding the foreign molecule to the functional group of the secondary silane, after which the silane part of this molecule provides the coupling to the silane part of the mercaptosilane on the metal particle surface. This procedure illustrates the potentiality for binding organic and inorganic species to the metal particles. Abundant possibilities can be imagined, taking into account the large amount of (commercially) available organosilanes, which can be combined with the metal colloids to form hybrid organic-inorganic materials.

Compared to other organosilane coupling agents, aminosilanes show a higher binding reactivity towards silica surfaces owing to the γ-amine group, which catalyzes the formation of siloxane bonds (*E.P. Plueddemann, Silane Coupling Agents; Plenum: New York, 1982*). Therefore they are of special interest to be used as the functional secondary silane compound. For the siloxane-bond formation, sufficient water needs to be present to protonate the amine and hydrolyze the alkoxy groups. Excess water, however, stimulates the formation of aminosilane multilayers. In case that one aims at binding a monolayer of aminosilane to the silane groups of the mercaptosilane on the metal particle surface, the reaction should be performed in the presence of

a small percentage of water. Usually, this percentage will be in the range of from 1 to 10% by weight.

The siloxane-bond formation that takes place in the coupling of functional secondary silanes, but also in the silane-cage formation of neighbouring silanes already present on the particle surface, is effectively catalyzed by amines, such as ethylamine, diethylamine, triethylamine, and ammonia (*J.P. Blitz, R.S. Shreedhara Murthy, and D.E. Leyden (1988) J. Colloid Interface Sci. 126: 387*).

Other secondary compounds that can be attached to the mercaptosilane-stabilized metal particle are silicates, aluminates, titanates, zirconates, or borates, like:

$Si(OR^1)_mR^2_{4-m}$; $Al(OR^1)_mR^2_{3-m}$; $Ti(OR^1)_mR^2_{4-m}$; $Zr(OR^1)_mR^2_{4-m}$; $B(OR^1)_mR^2_{3-m}$

wherein

R^1 = alkyl group or H;

R^2 = alkyl group with or without a functional group, or halogen;

m = a positive integer of from 0 to 4 (in case of Si, Ti and Zr compounds) or from 0 to 3 (in case of Al and B compounds).

They form, when $m \neq 0$, siloxane, silicoaluminate, silicotitanate, silicozirconate, and silicoborate bonds with the alkoxysilane groups of the metal-bound mercaptosilane. This is of special interest for the covalent incorporation of the metal particles in sol-gel materials.

FIELDS OF USE OF THE INVENTION

Applications in nano-structured materials

Future applications of quantum-sized metal nanoclusters can be thought of as nanoelectronic devices such as single-electron tunneling transistors, switches in the nanometer range, tunnel resonance resistors, non-linear optical devices, opto-electronic switches, and quantum lasers. The use of ultrasmall metal particles instead of semiconductor

material is of great advantage with respect to the miniaturization of such devices. Of special interest in these applications is the embedding of the particles in non-conducting host lattices, e.g. polymers, porous glasses, or zeolites (*R. Pelster, P. Marquardt, G. Nimtz, A. Enders, H. Eifert, K. Friedrich, F. Petzold (1992) Phys.Rev. B16: 8929*). Of the last-mentioned, lattices in which the metal particles have an ordered distribution with identical inter-particle distances (superlattices) are of special interest. The silane coat around the metal particles of the invention will show a high compatibility towards the inorganic lattices like zeolites and porous glasses and also will form covalent links to them, preventing particle coalescence.

Another option for fabrication is to press the ligand-stabilized particles into e.g. disks of quantum dots, or arrange them in one-dimensional arrays as quantum wires, where it is important that the ligands are not destroyed (causing particle coalescence) and that a constant inter-particle distance (smaller than about 2 nm) is maintained (*Schmid et al. (1994) US Patent no. 5,350,930*). For application in nano-structured materials, the mercaptosilane-functional silane coat (of controllable thickness) will keep the gold nanoparticles of the invention at a constant distance apart, being essential for a controlled single-electron-hopping conductivity. The rigidness of the siloxane cages around the metal particles of the invention will prevent the pressure forces to compress or even destroy the ligand shells, thereby changing ligand thickness.

The superlattices may well be suitable for another type of application, that is photonic crystals (*J.D. Joannopoulos, P.R. Villeneuve, S. Fan (1997) Nature 386: 143*). In miniaturization of integrated circuits the use of light instead of electrons becomes more and more popular. In structures with periodic variations in dielectric constant (the lattice constant of the photonoic crystal being comparable to the wavelength of light), a photonic bandgap exists of

frequencies which are forbidden. By introducing e.g. a line defect in the otherwise-perfect photonic crystal, this line can be used as a waveguide for light that propagates with a frequency within the bandgap (thus forbidden outside the waveguide in the perfect crystal). This way light can be directed specifically along the waveguide, confining light even around tight corners. The flexibility in tuning the frequency and localization properties of defects makes photonic crystals a very attractive medium for the design of novel types of optical filters, lasers and light-emitting diodes (LEDs). A new approach is the design of a 3D periodic lattice of isolated metallic regions within a dielectric host. The particles of the invention are of special interest because they can be incorporated chemically bound into a silica polymer network or colloidal crystal of highly mono-dispersed silica spheres.

An interesting application may be in energy-producing solar cells based on dye sensitization of large-bandgap semiconductor electrodes as described by Grätzel and co-workers (*B O'Regan and M. Grätzel (1991) Nature 353: 737*). First might be thought of the replacement of the dye with the metal nanoparticles of the invention, as described for CdS Q-particles (*H.Weller (1991) Ber. Bunsenges. Phys. Chem. 95: 1361*), where the CdS nanoparticles were deposited onto a porous TiO_2 electrode. The very small and silica-compatible metal particles will thoroughly penetrate the porous TiO_2 electrode by means of dipcoating or immersion. After that, they may be strongly attached to the TiO_2 network by ammonia-catalyzed hydrolysis and condensation reaction. After light absorption, the electrons generated in the metal Q-particle are injected into the TiO_2 substrate and the holes oxidize the electrolyte. Secondly, the metal particles of the invention may be combined with semiconductor particles to form covalently-attached sandwich colloids, that can be deposited in a porous electrode. Thirdly, on may think of covalently coupling one or more dye molecules to the metal

particles of the invention, thus combining the effects of the dye(s) and the metal Q-particle. The silane coat will give the metal nanoparticle a high photostability, a well-known problem in solar-cell applications.

Applications in (photo)catalysis

The importance of nanocolloids of catalytic metal is expressed by their high surface area and special (photo)-electronic properties at their surface. It has been found that e.g. by using Karstedt's catalyst $Pt[(vinyl)Me_2Si(OSi)_nOSiMe_2(vinyl)]_2$ (n=0-9) for catalyzing the hydrosilylation reaction, the molecular Pt complex turns into 1-5 nm colloidal Pt nanoparticles during the catalysis process *(L.N. Lewis, N. Lewis (1986) J.Am.Chem.Soc. 108: 7228)*. In fact, not the molecular Karstedt's Pt complex, but the colloidal Pt is thought to have the catalytic effect. With $Pt_{100-x}Au_x$ colloidal alloys interesting behavior has been observed towards hydrogenolysis and isomerization of, e.g., n-butane. Finely divided Pt supported on zeolites are very good isomerization catalysts, but also colloidal noble metal catalyst nanoparticles have been supported on ion-exchange resins, active carbon, intercalated clay compositions, colloidal graphite, alumina, and porous glasses. The silane-coated metal particles of the invention can be strongly anchored to the internal surface of the zeolite (alumino) silicate structure or a porous glass medium. The anchoring can be performed already during the synthesis of the zeolite or glass medium. The anchoring prevents the metal particles from aggregating, so that their catalytic activity is preserved.

Another application would be in photoelectrochemical solar cells e.g. for the production of hydrogen gas. Reactions are driven by minority charge carriers produced upon illumination. Instead of the classical procedures of metallizing semiconductor surfaces, electrocatalytic metal nanoparticles (e.g. Pt) could be deposited onto the semi-

conductor (e.g. p-GaAs particles) from a colloidal solution (*A. Meier, I. Uhlendorf, D. Meissner (1995) Electrochim. Acta 40: 1523*). This leads to the formation of multiple nano-contacts (MNCs), at the semiconductor/ electrolyte interface. By reduction of the size of the metal islands, the usually too small metal/semiconductor Schottky junction might be replaced by a much more effective barrier caused by the large metal/electrolyte interface. Combination of the metal nanoparticles of the invention with colloidal semiconductor particles (e.g. large-bandgap materials like TiO_2 and ZnO, or small-bandgap materials like CdS and Cd_3P_2) into sandwich colloids may well give even better results. Visible light excitation leads to an electron-hole pair in the small-bandgap particle of which the electron will immediately (picosecond range) transfer to the particle with the lowest conduction-band energy level. This may lead to charge-carrier separation, useful for photocatalysis. The particle size and surface properties of the metal particles of the invention may be tailored for this purpose easily and with a rich variety of both organic and inorganic compounds.

Particles of this invention which comprise Pt, Pd, Ag, Rh, Ru, Os, Ir are preferred, and especially Pt, Pd and/or Ag, more especially Pt and/or Pd, most of all Pt, are most preferred for these (photo)catalytic uses.

Applications in organic, inorganic and hybrid monoliths and coatings

The metal particles of the invention can be incorporated in colored glass. An optical glass can be prepared with controlled absorption properties, depending on the metal type, size, and uniformity of the incorporated metal particles. Especially important for a well-defined absorption spectrum of the glass is that the metal particles are non-aggregated. In the case of preparing the glass from a melt, the particles may be added to the melt. The silane coat around the particles will bind to the silane polymers and the particles

will be incorporated without aggregation. In the case of sol-gel processing from a solution, the silane coat of the particles will covalently attach to the silane (and/or titanate and/or zirconate and/or hybrid inorganic/organic) network. During drying and sintering of the sol-gel monolith, the particles will not coalesce. Also, the metal particles could be deposited on glass as a coating with certain reflection and absorption properties. Concerning coatings, also paints and lacquers can be loaded with the metal particles.

The metal nanoparticles can then be covalently attached to e.g. titania colloids before incorporating these in the paint. Another option is to use methacrylate-coated metal particles (see Example 6) to incorporate them in a methacrylate polymer network. In general, the metal nanoparticles can be cross-linked to plastic monomers in the production of e.g. polyethylene, polystyrene, PVC, polycarbonate, etc.

Applications as bioreagent-bound probes

The metal particles described in this paper can be conjugated to immunoreactive bioreagents such as antibodies, (strept)avidin and protein-A. As gold is the most beneficial biochemical probe for many reasons, this application section will further deal particularly with gold instead of the other metals. The main advantage of these conjugates over traditionally prepared immuno-gold reagents is that the coupling between gold and immunoreactive reagent is chemical instead of adsorptive. This means the stability is better and labeling better defined. The method to couple these gold particles relies on chemistry that is generally used to modify immunoreactive reagents with other proteins such as enzymes or avidin (*van Gijlswijk et. al. (1996) J. Imm. Meth. 189: 117-127; King et. al. (1979) J. Imm. Meth. 28: 201-210; Imagawa et. al. (1982) J. Applied Biochem. 4:41-47*).

The gold particles of the invention can be covalently coated with groups that are chemically reactive towards

bioreagents. These groups are e.g. sulfhydryls, maleimides, and iodoacetamides. The procedure is that first a molecule containing the bioreagent-reactive group (e.g. an N-hydroxy-succinimide ester of maleimide or iodoacetamide) is bound to the secondary silane compound (e.g. γ-aminopropyltriethoxy-silane), after which this is coupled via its alkoxysilane groups to the alkoxysilane groups of the gold-bound mercapto-silane. The silane reaction forms a siloxane cage around the gold particle. Bioreagent-functional gold particles that are most used in the examples are those coated with S-acetylthio-acetate groups, which have a protected sulfhydryl group (*Duncan et al. (1983) Anal. Biochem. 132:68-74*). These particles are deprotected prior to use, after which the sulfhydryl activity remains unchanged during long-time storage of the particles. Upon coupling of the (deprotected) gold particles to the protein, the protein must contain a sulfhydryl-reactive group. Either maleimides or iodoacet-amides are used for this purpose in the examples, implying that the gold particles are labeled to the proteins by virtue of a thiolether bond. Maleimides or iodoacetamides can be introduced to immunoreactive proteins by incubating these proteins with N-hydroxysuccinimide esters of maleimide or iodoacetamide. A fraction of the lysine aminoacid residues of the protein react with the ester and the maleimide or iodoacetamide is bound through a stable amide bond.

Similar chemistry can be applied to couple the gold particles of the invention to amino-labeled oligonucleotides and nucleotides. Because of the relative inertness of the particles described here, they allow applications such as in-situ hybridization with probes directly labeled with gold particles. The silane cage-like coat of the gold particles of the invention gives them a high chemical and temperature stability. Other chemically-reactive gold particles (e.g. those stabilized with triphenylphosphine groups (*Hainfeld et al. (1994) US Patent no. 5,360,895*)) are less heat stable and consist of chemically less stable bonds between the gold

particle and its functional group. Especially chemicals such as thiols, urea, or formamide, being reagents generally applied in different hybridization assays, may induce particle degradation. Also chemically-reactive gold particles stabilized with organothiols (*Hainfeld et al. (1996) US Patent no. 5,521,289*) will not have the inertness of the silane-stabilized gold particles of the invention.

Another advantage of these silane-stabilized gold particles is the easy ability to bind more than one molecule simultaneously to the mercaptosilane shell of each gold particle. For instance, besides protected sulfhydryl groups for the bioreagent coupling, also fluoresceins and/or biotin molecules can be attached to the same particle, rendering a multi-functional gold probe. The fluorochrome-labeled gold particles serve as easily detectable labels to check their conjugation to high molecular weight biomolecules, but they can also be used for immunocytochemical analysis. Immunochemical labeling prepared for electron microscopy can first easily be confirmed by fluorescence microscopy to obtain information at the cellular level before EM is applied for information at the sub-cellular level. Concerning the fluorescent signal, the siloxane cage has an insulating effect on the electronic interaction between the energy levels of the gold (quantum-) particle and those of the fluorescent molecules. This way, quenching of the fluorescence signal is reduced, a problem especially serious for non-covalently bound gold probes.

Hapten-labeled gold particles can be used for similar experiments and furthermore the hapten can function as a tool in the separation of gold-labeled immunoreagents from non-gold-labeled counterparts. It is also possible to introduce more than two labels on the surface of the gold particle, allowing a combination of reactive groups for conjugation, chemically-cleavable haptens for purification (see Examples 14 and 15), and fluorochromes for the easy and sensitive confirmation of protein labeling and immunochemistry.

Generally, one can use the gold labels of the invention bound to immunoglobulins, lectins, hormones, etc. for immuno-labeling and effector-labeling in high-resolution (electron microscopic) localization studies. For localization of DNA or RNA one can apply hybridization of gold-labeled oligo-nucleotides for high-resolution gene (transcript) mapping on chromosomes or DNA spreads. One can study intracellular transport phenomena by recording the gold label uptake by e.g. vesicles, or study molecular interactions by injecting a gold-marked protein to map its intracellular target(s). One might establish the localization of a certain protein in large protein complexes such as viruses, proteosomes, ribosomes, ribosomal nuclear particles, and nucleosomes, by labeling that specific protein with gold.

For application in cancer therapy, radioactive gold conjugates can be targeted to cancer cells. Non-radioactive gold particles, targeted to cancer cells, can then be activated, e.g. by heating them with an appropriate energy beam.

The full content of all references mentioned herein is incorporated herein by reference.

EXAMPLE 1

Preparation of MPS-stabilized gold sols in ethanol of different particle sizes from 5 nm to smaller

Preparation

(1) The gold sols were prepared in absolute ethanol (Nedalco) at room temperature by the reduction of hydrogen tetrachloroaurate(III) (prepared with $HAuCl_4 \cdot (H_2O)_y$, Janssen, 49 wt.% Au) with sodium borohydrate ($NaBH_4$, dissolved in twice-distilled water) in the presence of γ-mercaptopropyl-trimethoxysilane (MPS, Fluka, >97% purity) as a particle stabilizer (see Figure 1a). To 10.0 ml of ethanol were successively added 1.00 mL of a *x* g/L MPS solution in ethanol and 200 μL 13.5 mM aqueous $HAuCl_4$ solution. To change the $HAuCl_4$:MPS molar ratio, the concentration of the MPS solution

was varied between x = 80 mg/L (0.41 mM) and x=10.0 g/L (50.9 mM). To the fresh $HAuCl_4$-MPS mixture, 200 µL of a 100 mM $NaBH_4$ aqueous solution was added in one portion under vigorous stirring. A $NaBH_4$:$HAuCl_4$ molar ratio beyond seven ensured complete and fast reduction. Immediately, a colouring appeared which depended on the $HAuCl_4$:MPS ratio. This ratio and an identification of the solvent used are incorporated in brackets in the sol codes Au(Et,6.62) to Au(Et,0.053). The aqueous $HAuCl_4$ stock solution remained (visibly) unchanged upon storage at room temperature during several months. Gold sols prepared with a three months old $HAuCl_4$ stock solution did not show any difference in comparison to sols prepared with a fresh solution with respect to sol colour, absorption spectra, and electron micrographs.

(2) Gel filtration was performed to remove free MPS from the sols. For this a Sephadex LH-20 (Pharmacia Biotech) column was used with ethanol as the eluent. Sephadex LH-20 in ethanol shows a void volume percentage of 32% and, for polyethylene glycol, an exclusion limit at a molecular weight of about 4000 g/mol. The sol coded Au(Et,0.529) migrated as a narrow brown band of constant width. The relative elution volume of ethanol needed for the particles to migrate through the column was roughly in the order of 30% of total column volume, implying that the MPS-stabilized gold particles elude at void volume.

Results

(1) Table 1 shows the results of the reduction by $NaBH_4$ of a series of $HAuCl_4$-MPS mixtures with varying $HAuCl_4$:MPS ratio. Upon adding little MPS, corresponding to a $HAuCl_4$:MPS ratio higher than about six, all the gold immediately flocculated and formed a (purple-)black sediment. Upon adding more MPS, stable sols were formed which showed a trend in their colour from wine-red for sol Au(Et,5.29) to yellow-brown for sol Au(Et,0.106).

Table 1. Alcosols of MPS-stabilized gold particles [a].

Sample Code [b]	Appearance	D_{TEM} (nm)
Au(Et,6.62)	black sediment	aggregated
Au(Et,5.29)	wine-red sol	3 - 5
Au(Et,1.70)	orange sol	2 - 3
Au(Et,0.529)	light brown sol	1.0 - 1.5
Au(Et,0.106)	yellow-brown sol	≤ 1.0
Au(Et,0.053)	light yellow solution	?

[a] Appearances of the sols and estimated sizes of their particles. In all sols [Au] = 1.75×10^{-4} M.

[b] The solvent (Et=ethanol) and the $HAuCl_4$:MPS molar ratio are shown in brackets.

It had no effect on the particle size or sol stability whether the $NaBH_4$ reductor solution was added to the $HAuCl_4$-MPS mixture at once or in a sequence of smaller portions. After every portion added, a certain corresponding amount of gold particles was formed, expressed by an increasing intensity of the sol colour. The UV-visible absorption spectrum preserved its trend, and the gold particles were as uniform as in the case that all $NaBH_4$ was added at once. In fact, only the initial $HAuCl_4$:MPS ratio determined the particle size and uniformity. To obtain a sol that retains its stability in time, however, it is essential that the total molar amount of added $NaBH_4$ is about seven times the amount of $HAuCl_4$.

For the preparation of sol Au(Et,0.106), the $NaBH_4$ was not added immediately after mixing the MPS and $HAuCl_4$. A stable gold sol was only obtained after the $HAuCl_4$-MPS mixture first had been stirred for about ten minutes until it had changed from yellow to colourless. This colour change also took place for the other $HAuCl_4$:MPS ratios and took more time for increasing $HAuCl_4$:MPS ratio. The absorption spectrum was recorded in time for a $HAuCl_4$-MPS mixture of $HAuCl_4$:MPS ratio 0.529. After mixing the 1.00 mL 1.0 g/L MPS and 200 µL

13.5 mM $HAuCl_4$ in ten mL of ethanol, the absorption band at 320 nm, being characteristic for $AuCl_4^-$, gradually decreased in intensity (without shifting its position) until it completely disappeared after hundred minutes. The solution then was colourless. Apparently, the $AuCl_4^-$ complex gradually changed into a MPS-gold complex. It may be possible that the thiol group of MPS, by oxidizing to a disufide, reduces the Au(III) from the tetrachloroaurate to Au(I) which complexes with MPS. The addition of $NaBH_4$ then further reduces this Au(I) complex to form MPS-capped gold particles.

The addition of $NaBH_4$ to a $HAuCl_4$-MPS mixture (immediately after mixing) of a very low $HAuCl_4$:MPS ratio of 0.053, yielded a light yellow solution (sol Au(Et,0.053)). In the transmission electron microscope, no particulate gold was observed in bright field. We do not have a clear understanding of the gold species in this solution. Possibly some kind of gold-MPS-chloride complex is present.

(2) Bright-field electron-microscopic images were made with a Philips EM-420 transmission electron microscope (TEM) operated at 80 kV. High-angle-annular-dark-field images were made with a Philips CM-200 scanning-transmission electron microscope (HAADF-STEM) equipped with a field emission gun and operated at 200 kV. Sample preparation for TEM and STEM was performed by dipping a carbon-supported 400-mesh copper electron-microscopy grid in the alcosol, draining the excess alcosol from the grid, and drying in the air.

The particle size determinations by electron microscopy (see Table 1) reveal that a decrease of the $HAuCl_4$:MPS ratio causes a decrease of the particle diameter from about 3-5 nm for sol Au(Et,5.29) to about 1 nm or even smaller for sol Au(Et,0.106). The gold particles of sol Au(Et,5.29), visualized by TEM (Figure 2a), are quite non-isometric and non-uniform in size. The aggregates of these particles on the micrograph are due to drying effects on the EM grid. The TEM micrograph of Au(Et,0.529) (Figure 2b) shows nanometer-sized gold particles in an aggregate structure in which the indivi-

dual particles cannot be identified anymore. With HAADF-STEM on the same sample grid, the individual Au(Et,0.529) particles can be visualized (Figure 2c). Due to the lack of a clear correlation between the darkfield representation of the particles and their actual size, the particle size can only be estimated to be in the range of 1.0 to 1.5 nm. Figure 2c illustrates that the gold particles, whatever their precise diameters, have quite uniform dimensions. The micrograph also suggests that the particles have an isometric shape. The particles of Au(Et,0.106) show a dark-field intensity which is less bright than that of the Au(Et,0.529) particles. Therefore, they are probably even smaller than those of Au(Et,0.529), and of subnanometer size.

(3) Near-ultraviolet-visible absorption spectra were recorded for wavelengths between 290 nm and 700 nm with a Spectronic 200 UV spectrophotometer. The measurements reveal that the wine-red colour of sol Au(Et,5.29) is connected to the presence of a broad rudimentary plasmon absorption band at 520 nm wavelength. This band, being typical for particulate gold sols, is absent in the spectrum of the light-brown sol Au(Et,0.529). The disappearance of the plasmon band from the spectrum can be attributed to quantum-size effects accompanying the reduction of the gold particle dimensions. Duff et al. (*D.G. Duff, A. Baiker, and P.P. Edwards (1993) Langmuir 9: 2301*) found the plasmon band present for a scarlet sol of 4.3 nm gold particles, and its near absence (shoulder) for an orange-brown sol of 1.4 nm gold particles. In view of the colour and spectrum of sol Au(Et,0.529), our particle size estimation of 1.0 to 1.5 nm seems to be plausible.

EXAMPLE 2

Modification of the MPS-stabilized gold particles with APS

Preparation of an APS-modified gold sol

One mL of sol coded Au(Et,0.529) was immediately after its preparation purified by gel filtration over a Sephadex LH-20 column with ethanol as eluent. About 3 mL sol was collected. To this were added 100 µL of a 10.0 g/L γ-amino-propyltriethoxysilane (APS, Janssen, 99% purity) solution in absolute ethanol and 250 µL of ammonia (Merck, p.a., 25% NH_3). This was stirred at room temperature for 15 minutes. Then again, 1 mL of this sol was purified from ammonia and excess APS over the column in ethanol (final code Au(Et,0.529)-APS). (The alcosol Au(Et,0.529)-APS could be transformed to an aquasol by dialysis against demineralized water while retaining its colloidal stability.)

Results

(1) Investigation of the (purified) light-brown alcosol Au(Et,0.529)-APS by HAADF-STEM showed that the gold cores still had the same degree of homogeneity as before the modification with APS. The modification neither resulted in aggregation of the particles; particle doublets and triplets probably were formed during the drying of the sol on the EM grid.

(2) Fourier-transform infrared (FT-IR) spectroscopy was performed on a Perkin Elmer System 2000 FT-IR spectrometer with an attenuated total reflection unit equiped with a ZnSe crystal (SpectraTech). The sample solution was deposited on the crystal and dried by evaporating its solvent in the air at room temperature. Besides Au(Et,0.529)-APS, also free MPS and APS were measured for comparison (applied as 10 g/L solutions in ethanol).

The proof that APS is indeed bound to the Au(Et,0.529)-APS particles is the presence in their spectrum of a strong C-N band at 1074 cm^{-1}. The spectrum of Au(Et,0.529)-APS

further shows a strong band of methoxysilane (2961 cm^{-1}) coming from MPS and strong bands of siloxane (Si-O-Si) bonds (1094 and 1026 cm^{-1}). In the Au(Et,0.529)-APS spectrum, any bands of the SH and NH_2 groups of MPS and APS, respectively, are not visible. The already very weak band of the SH group of MPS has disappeared due to its chemisorption on the gold particle, the NH_2 band probably has disappeared in the background due to hydrogen bonding to silanols. A peculiar band in the Au(Et,0.529)-APS spectrum is a strong peak at 1730 cm^{-1}, which can only be assigned to a carbonyl stretching vibration of an ester compound. The band seems too strong to be imputed to a contamination. Probably, the gold surface (possibly in co-operation with the amine group) has some catalyzing effect on the oxidation of alcohol to ester.

EXAMPLE 3

Preparation of fluorescein-covered gold particles

For this, fluorescein isothiocyanate (FITC, Sigma) was first coupled to the APS (FITC forms an isothiourea linkage with the APS amine) by bringing 50 mM of each in ethanol and stirring for two hours (APS-FITC). To 11.4 mL of an unpurified sol coded Au(Et,0.529) were added 100 µL of the 50 mM APS-FITC solution and 500 µL ammonia (25% NH_3). This mixture was heated at 60°C for four hours. Then, this sol was transferred to water and purified from ammonia and excess APS-FITC by dialysis in a cellulose tube against demineralized water for one week until no FITC could be visually observed in the supernatant. It showed a dominant yellow-green fluorescent colouring of the FITC.

EXAMPLE 4

Preparation of MPS-stabilized Au, Pt, Pd and Ag sols in several solvents

(1) To 11.0 ml of solvent were subsequently added 200 µl of a 0.1% (w/v) MPS solution in ethanol, and 400 µl of a 0.01 M metal salt solution. After 1 min of stirring, the metal was

reduced with 400 µl of a 0.10 M $NaBH_4$ solution in H_2O. The following solvent/metal salt combinations were successful to prepare metal sols.

Au sols: EtOH, MeOH, H_2O, DMSO, DMF, aceton / $HAuCl_4$ (dissolved in H_2O, EtOH, or DMSO); After 20 h of stirring, the aceton sol showed some flocculated material but still contained a stable sol above it. After purification over Sephadex LH-20/aceton eluent the aceton sol showed a long-time stability.

Pt sols: EtOH, MeOH, H_2O, DMSO, DMF / H_2PtCl_6 (dissolved in H_2O, DMSO or EtOH);

Pd sols: EtOH, MeOH, H_2O, DMSO, DMF / $PdCl_2$ (dissolved in EtOH in the presence of 0.10 M HCl)

Ag sols: DMSO, DMF / $AgNO_3$ (dissolved in H_2O); the Ag particles are quickly oxidized in excess H_2O or EtOH.

(2) The sols were purified with a Sephadex LH-20 column with eluent EtOH, MeOH, DMSO, DMF, or aceton. The metal particles traveled in the void volume (exclusion limit of Sephahex LH-20 is ± MW 4000). The sols in H_2O or DMSO could be purified with Sephadex G-50, Sephadex G-75, or Sephadex G-100 with H_2O or DMSO eluent. In the Sephadex G-series the metal particles should be separated on size.

EXAMPLE 5

Preparation of MPS-stabilized bimetallic Au-Ag sols

Preparation

Sol $Au_{50}Ag_{50}$(Et,7.56): To 11.0 ml of ethanol were subsequently added 1.0 ml of a 0.01% (w/v) MPS solution in ethanol, 200 µl of a 0.01 M $HAuCl_4$ solution in H_2O, and 100 µl of a 0.02 M $AgNO_3$ solution in H_2O. After 1 min of stirring, the metal was reduced with 200 µl of a 0.10 M $NaBH_4$ solution in H_2O.

Sol $Au_{50}Ag_{50}$(Et,0.756): as above but applying a 0.1% (w/v) MPS solution in ethanol.

Results

$Au_{50}Ag_{50}$(Et,7.56) was a dark purple-red sol of estimated particle size 5 nm and $Au_{50}Ag_{50}$(Et,0.756) was a dark brown-red sol of estimated particle size 2 nm.

Conclusion

Bimetallic particles can be stabilized with MPS and their size can be controlled by the MPS-to-total metal ion ratio.

EXAMPLE 6

Preparation of methacrylate-coated gold particles, applicable as alcosol and as soluble powder

Preparation

(1) Sol Au(Et,0.378)-TPM was prepared as follows. To 10.0 ml of ethanol were subsequently added 1.0 ml of a 0.1% (w/v) MPS solution in ethanol, and 200 µl of a 0.01 M $HAuCl_4$ solution in H_2O. After 1 min of stirring, the metal was reduced with 200 µl of a 0.10 M $NaBH_4$ solution in H_2O. Then were added 500 µl of ammonia (25% NH_3) and 1.0 ml of a 0.1% (w/v) solution of γ-trimethoxysilyl propyl methacrylate (TPM) in ethanol. The mixture was stirred for 24 h at RT.

Drying and redissolving

The ethanolic solvent was slowly evaporated by drying in air at 70°C until a dry brown solid remained sticking to the bottom of the glass container. By adding ethanol (about 5 ml) at RT and gently shaking by hand, the solid immediately dissolved to form a clear brown sol.

Conclusion

Methacrylate-coated ultrasmall-gold particles can be processed in powder form without irreversible particle coalescence. This may have important benefits for their application.

EXAMPLE 7

Preparation of SATA-functional gold sol in DMSO

Preparation

(1) Analogous to the preparation in ethanol, a gold sol was prepared in DMSO as follows: To 11.0 ml of DMSO under stirring were subsequently added: 200 µl of a 0.10% MPS solution in ethanol, and 400 µl of a 10 mM $HAuCl_4$ solution in ethanol. This light green-yellow solution was stirred for 1 min, after which gold reduction was achieved by adding 400 µL of a 100 mM $NaBH_4$ solution in H_2O (twice distilled). The sol color turned brown (sol code Au(Dm,3.78)).

(2) APS was reacted with SATA by adding 44.3 mg of APS and 46.2 mg of SATA to 1.0 ml of DMSO, resulting in a 200 mM APS-SATA solution in DMSO.

(3) After 3 min of stirring sol Au(Dm,3.78), subsequently were added: 100 µL of a 200 mM APS-SATA solution in DMSO and 500 µL of triethylamine (TEA, 99%). This solution was stirred for about 20 h. Then it was purified by gel filtration as follows. A volume of 1.0 ml was loaded onto a 5 ml Sephadex LH-20 in DMSO column and eluded with DMSO (sol code Au(Dm,3.78)-SATA). The sol travelled at void volume as a narrow brown band.

Results

(1) By applying Ellmanns Reagent to the different Au(Dm,3.78)-SATA fractions collected from the column, it was confirmed that the purified Au(Dm,3.78)-SATA particles had SATA groups bound their surface.

(2) The Au(Dm,3.78)-SATA fractions were spotted (1 µl) on Hybond-N^+ nylon transfer membrane (Amersham) and dried at 60°C. Then the spots were silver-enhanced with Danscher's silver enhancement solution (see *G. Danscher (1983) J. Histochem. Cytochem. 31:1394*). The spots enhanced well to brown spots of intensity according to their gold concentration.

EXAMPLE 8

Conjugation of SATA-functional gold particles to Goat IgG anti Rabbit IgG; SMCC method

Conjugation

(1) 3 mg Goat IgG anti Rabbit IgG (GαR, Rockland Laboratories) was dissolved in 600 µl 1×NHS buffer. To this, 13.5 µl of a 5 mg/ml SMCC (Pierce) solution in DMSO was added (67 µg SMCC). This GαR-SMCC mixture was incubated for 1 h at 30°C.

(2) The GαR IgG / SMCC mixture was concentrated to 200 µL using a c30-filter (Amicon) and further purified using a 2 ml Sephadex G-50 Fine column with 1×MEI buffer as the eluent. The fraction eluting at 0.6-1.2 ml was pooled and divided over 3 fractions of 0.2 ml of maleimide-functional GαR IgG each (GαR-MEI).

(3) To 350 µl of Au(Dm,3.78)-SATA (in DMSO) were added: 500 µl of H_2O, 200 µl of 5×SATA buffer, and 115 µl of a 0.5 M $HONH_2$ solution in 1×SATA buffer. This was incubated for 20 min at 30°C to produce unprotected Au(Dm,3.78)-SH.

(4) The three 0.2 ml volumes of maleimide-functional GαR IgG portions (see (2)) were incubated with different amounts of Au(Dm,3.78)-SH:

1. 0.2 ml GαR-MEI + 0.1 ml Au(Dm,3.78)-SH
2. 0.2 ml GαR-MEI + 0.3 ml Au(Dm,3.78)-SH
3. 0.2 ml GαR-MEI + 0.75 ml Au(Dm,3.78)-SH

These were incubated for 2 h at RT. Then the solutions were diluted to 1.0 ml with 1×PBS buffer to yield a 1 mg/ml IgG solution (coded GαR-Au).

Spot test on filter

(1) A series of dilutions were prepared of Mouse IgG in a 0.1% BSA-containing 1×PBS blocking buffer. From these dilutions, 300, 100, 30, 10, 3, 1, 0.3, 0.1, 0.03, and 0.0 ng Mouse IgG were spotted (1 µl) onto four Hybond C Extra transfer membrane filters (Amersham).

(2) After drying in air, the filters were preincubated in TNB buffer for 30 min at RT.

(3) Then, 2 µg/ml Rabbit IgG anti Mouse IgG (RαM, Sigma) in TNB buffer was applied. After 30 min at RT, the filters were washed 3 times for 5 min in TNT buffer.

(4) Then, three filters were incubated with the three GαR-Au conjugates (see Conjugation (4)), 1:200× diluted to 5 µg/ml IgG in TNB buffer. As a control experiment, the fourth filter was incubated with GαR-alkaline phosphatase (2 µg/ml, Sigma). After 30 min at RT all filters were washed 3 times for 5 min in TNT buffer and finally one in twice-distilled H_2O.

(5) Visualization of alkaline phosphatase was performed by applying NBT/BCIP (1 component mix, Rockland) to the filter for 2 h. Visualization of the gold labels was performed by incubating the three different filters with Danscher's silver enhancer for 2 h.

Results

All three GαR-to-gold conjugates (see Conjugation (4)) provided specific staining of Mouse IgG. No significant difference was observed in the staining results of the three different GαR-to-gold conjugates, which means that 1 mg IgG is optimally labeled with ± 0.1 ml Au(Dm,3.78)-SATA goldsol. Their detection limit was found to be ± 3 ng Mouse IgG. The alkaline phosphatase method was found to detect ± 30 pg Mouse IgG. The example clearly shows that maleimide-activated IgG can be used in combination with APS-SATA and MPS-functionalized gold particles to prepare IgG-gold conjugates.

EXAMPLE 9

Conjugation of SATA-functional gold particles to Goat IgG anti Mouse IgG; SIAB method

Conjugation

(1) To 1 mg Goat IgG anti Mouse IgG (GαM, Rockland Laboratories) in 0.2 ml 1×NHS buffer was added 5 µl of a

10 mg/ml SIAB (Pierce) solution in DMSO (50 µg SIAB). This mixture was incubated for 1 h at RT.

(2) The mixture was purified using a 2 ml Sephadex G-50 Fine column. Fractions eluting at 0.6-1.2 ml were pooled and divided into two portions of 0.3 ml iodoacetamide-functional GαM (GαM-IAA).

(3) As a control experiment, GαM was labeled with horseradish peroxidase (HRP): to 50 µl of a 20 mg/ml solution of HRP-SATA (*R.P.M. van Gijlswijk, D.J. van Gijlswijk-Janssen, A.K. Raap, M.R. Daha, and H.J. Tanke (1996) J. Immun. Meth. 189: 117*) in 1×SATA buffer (1 mg HRP) was added 5 µl of a 0.5 M $HONH_2$ solution in 1×SATA buffer. This was incubated for 20 min at 30°C to produce unprotected HRP-SH. To 0.1 ml Au(Dm,3.78)-SATA (in DMSO) was added 40 µl of a 0.5 M $HONH_2$ solution in 1×SATA buffer. This was incubated for 20 min at 30°C to poduce unprotected Au(Dm,3.78)-SH.

(4) To one 0.3 ml portion of GαM-IAA (0.5 mg IgG) was added 55 µl HRP-SH and to the other 0.3 ml portion of GαM-IAA was added 140 µl Au(Dm,3.78)-SH. These were incubated for 4 h at 30°C (coded GαM-HRP and GαM-Au).

Spot test on filter

(1) A series of dilutions were prepared of Mouse IgG in a 0.1% BSA-containing 1×PBS blocking buffer. From these dilutions, 300, 100, 30, 10, 3, 1, 0.3, 0.1, 0.03, and 0.0 ng Mouse IgG were spotted (1 µl) onto two Hybond C Extra transfer membrane filters (Amersham).

(2) After drying in air, the filters were preincubated in TNB buffer for 30 min at RT.

(3) Then, one filter was incubated with 2 µg/ml GαM-Au conjugates, 1:1000× diluted to 2 µg/ml IgG in TNB buffer. As a control experiment, the second filter was incubated with GαM-HRP (5 µg/ml). After 30 min at RT, the filters were washed 3 times for 5 min in TNT buffer and finally one in twice-distilled H_2O.

(4) Visualization of HRP was performed by applying DAB/Ni (*R.P.M. van Gijlswijk, D.J. van Gijlswijk-Janssen, A.K. Raap, M.R. Daha, and H.J. Tanke (1996) J. Immun. Meth. 189: 117*) to the filter for 20 min. Visualization of the gold labels was performed by incubating the three different filters with Danscher's silver enhancer for 2 h.

Results

The GαR-to-gold conjugate provided specific staining of Mouse IgG, with a detection limit of ± 15 ng Mouse IgG. The HRP method was found to detect ± 30 pg Mouse IgG. The example shows that iodoacetate-activated IgG can be used in combination with APS-SATA and MPS-functionalized gold particles to prepare IgG-to-gold conjugates.

EXAMPLE 10

Conjugation of SATA-functional gold particles to Avidin-D; SIAB method

Conjugation

(1) To 1 mg avidin-D (Vector Laboratories) in 0.2 ml 1×NHS buffer was added 17 µl of a 10 mg/ml SIAB (Pierce) solution in DMSO (170 µg SIAB). This mixture was incubated for 1 h at RT.

(2) The mixture was purified using a 2 ml Sephadex G-50 Fine column. Fractions eluting at 0.6-1.2 ml were pooled and divided into two portions of 0.3 ml iodoacetamide-functional avidin-D (AvidinD-IAA).

(3) As a control experiment, avidin-D was labeled with horseradish peroxidase (HRP): to 50 µl of a 20 mg/ml solution of HRP-SATA (*R.P.M. van Gijlswijk, D.J. van Gijlswijk-Janssen, A.K. Raap, M.R. Daha, and H.J. Tanke (1996) J. Immun. Meth. 189: 117*) in 1×SATA buffer (1 mg HRP) was added 5 µl of a 0.5 M $HONH_2$ solution in 1×SATA buffer. This was incubated for 20 min at 30°C to produce unprotected HRP-SH. To 0.1 ml Au(Dm,3.78)-SATA (in DMSO) was added 40 µl of a

0.5 M $HONH_2$ solution in 1×SATA buffer. This was incubated for 20 min at 30°C to poduce unprotected Au(Dm,3.78)-SH.

(4) To one 0.3 ml portion of AvidinD-IAA (0.5 mg avidin-D) was added 55 µl HRP-SH and to the other 0.3 ml portion of AvidinD-IAA was added 140 µl Au(Dm,3.78)-SH. These were incubated for 4 h at 30°C (coded AvidinD-HRP and AvidinD-Au).

Spot test on filter

(1) A series of dilutions were prepared of Mouse IgG in a 0.1% BSA-containing 1×PBS blocking buffer. From these dilutions, 300, 100, 30, 10, 3, 1, 0.3, 0.1, 0.03, and 0.0 ng Mouse IgG were spotted (1 µl) onto two Hybond C Extra transfer membrane filters (Amersham).

(2) After drying in air, the filters were preincubated in TNB buffer for 30 min at RT.

(3) Then, the filters were incubated with 2 µg/ml of a biotinylated Goat IgG anti Mouse IgG (GαM-Biotin, Sigma) in TNB buffer. After 30 min at RT, the filters were washed 3 times for 5 min in TNT buffer and finally one in twice-distilled H_2O.

(4) Then, one filter was incubated with AvidinD-Au, 1:500× diluted to 5 µg/ml avidin-D in TNB buffer. As a control experiment, the second filter was incubated with GαM-HRP (5 µg/ml). After 30 min at RT, the filters were washed 3 times for 5 min in TNT buffer and finally one in twice-distilled H_2O.

(5) Visualization of HRP was performed by applying DAB/Ni (*R.P.M. van Gijlswijk, D.J. van Gijlswijk-Janssen, A.K. Raap, M.R. Daha, and H.J. Tanke (1996) J. Immun. Meth. 189: 117*) to the filter for 20 min. Visualization of the gold labels was performed by incubating the three different filters with Danscher's silver enhancer for 2 h.

Results

The avidin-D-to-gold conjugate provided specific staining of Mouse IgG, with a detection limit of ± 3 ng Mouse

IgG. The HRP method was found to detect ± 10 pg Mouse IgG. The example shows that iodoacetate-activated avidin-D can be used in combination with APS-SATA and MPS-functionalized gold particles to prepare avidin-D-to-gold conjugates.

EXAMPLE 11

Preparation of FITC-SATA-functional goldsol in DMSO

Preparation

(1) To 11.0 ml of DMSO under stirring were subsequently added: 200 µl of a 0.10% MPS solution in ethanol, and 400 µl of a 10 mM $HAuCl_4$ solution in ethanol. This light green-yellow solution was stirred for 1 min, after which gold reduction was achieved by adding 400 µL of a 100 mM $NaBH_4$ solution in H_2O (twice distilled). The sol color turned brown (sol code Au(Dm,3.78)).

(2) 200 mM APMS-SATA and 50 mM APMS-FITC solutions in DMSO were prepared by stoichiometrically adding the reagents APMS, SATA and FITC and letting them react for one day at RT.

(3) After 3 min of stirring sol Au(Dm,3.78), 500 µL of triethylamine (TEA, 99%) was added and the sol was stirred for 1 h at RT. Then subsequently were added: 50 µL of the 200 mM APMS-SATA solution in DMSO and 50 µL of the 50 mM APMS-FITC solution in DMSO (SATA:FITC=4:1). This solution was stirred for about 1 h at RT. Then, purification was performed by gel filtration over a Sephadex LH-20 column, eluded with DMSO (sol code Au(Dm,3.78)-FITC-SATA).

Result

The Au(Dm,3.78)-FITC-SATA fractions were spotted (1 µl) on Hybond-N^+ nylon transfer membrane (Amersham) and dried at 60°C. Then the spots were silver-enhanced with Danscher's silver enhancement solution. The silver enhancement is more strongly than that for Au(Dm,3.78)-SATA in Example 7.

EXAMPLE 12

Conjugation of FITC-SATA-bifunctional gold particles to Avidin-D; SIAB method

Conjugation

Method analogous to that in Example 10 (Conjugation section). Sol code AvidinD-FITC-Au.

Spot test on filter

(1) A series of dilutions were prepared of Mouse IgG in a 0.1% BSA-containing 1×PBS blocking buffer. From these dilutions, 300, 100, 30, 10, 3, 1, 0.3, 0.1, 0.03, and 0.0 ng Mouse IgG were spotted (1 µl) onto Protran Nitrocellulose membrane filters (Schleicher & Schuell).

(2) After drying in air, the filters were preincubated in TNB buffer for 20 min at RT.

(3) Then, the filters were incubated with biotinylated Sheap IgG anti Mouse IgG (ShαM-Biotin), 1:1000× diluted in TNB buffer. After 30 min at RT, the filters were washed 3 times for 5 min in TNT buffer and finally once in twice-distilled H_2O.

(4) Then, a filter was incubated with AvidinD-FITC-Au, 1:50× diluted to 50 µg/ml avidin-D in TNB buffer. As a control experiment, the second filter was incubated with AvidinD-HRP. After 30 min at RT, the filters were washed 3 times for 5 min in TNT buffer and finally once in twice-distilled H_2O.

(5) A second filter which had been incubated with AvidinD-FITC-Au was subjected to incubation with HRP-labeled Sheep IgG anti FITC (ShαFITC-HRP) for 30 min at RT.

(6) Visualization of the gold and HRP labels was performed as described in Example 10.

Result

Before silver enhancement, the FITC-and-avidin-D-to-gold conjugate showed a Mouse-IgG-specific fluorescence signal (already seen by the naked eye) and after silver enhancement

a Mouse-IgG-specific staining of with a detection limit of ± 1 ng Mouse IgG. The HRP method was found to detect ± 1 pg Mouse IgG. The example shows that the FITC-avidin D bifunctional gold particle works well in the sense that is a specific label for both fluorescence and gold. The labeling with ShαFITC-HRP gave only minor DAB/Ni staining. Apparently, the FITC molecules in the silane-surface layer of the gold particle are situated in such an 'embedded' state that the anti-FITC IgG molecules cannot reach it.

EXAMPLE 13

In-situ hybridization using avidin D-gold conjugate

Metaphase probes

(1) Standard metaphase probes were cultivated on glass slides (colcemid, methanol/acetic acid (3:1)-fixed). After 24 h drying kept in 70 % ethanol at 4°C.

(2) After dehydration, the glass slides were dried for ± 10 min at RT. Then they were incubated with a 100 µg/ml Rnase solution in 2×SSPE buffer for 20 min at RT to break down cytoplasmic RNA. Then, the glasses were washed three times 3 min in PBS buffer and after that 2 min in twice-distilled water. Then, incubation with 0.05% pepsine in 0.01 M HCl for 10 min at 37°C to increase accessibility of the nucleus. Then, washing two times 3 min in PBS buffer. Then, after-fixation in 1% formaldehyde in PBS buffer/50 mM $MgCl_2$ for 10 min at RT. Then, another two times 3 min washing in PBS buffer and dehydration.

5'HRP-labeled oligo-probe hybridization and labeling with avidin-D-gold

(1) The metaphases were denaturated in 70% formamide in 2×SSC buffer for 10 min at 80°C. Then, they were dehydrated in an alcohol concentration series and dried in the air. The metaphases were hybridized left with 1q12-HRP (1:200× diluted in ureum hybmix), and right with Yq12-HRP (1:400× diluted in

ureum hybmix), for 16 h at 37°C. The glass slides were washed three times 5 min in 3 M ureum/1×SSTE buffer/0.1% Tween 20 and after that 5 min in PBS buffer.

(2) The slides were incubated with biotin-tyramide, 1:300×diluted in 1×amplification buffer (purchased from NEN) with 12.5% dextrane sulfate. The slides were washed three times 3 min in TNT buffer.

(3) The slides were incubated for 30 min with avidinD-Au (from Example 10), 1:50× diluted in TNB buffer. Then, they were washed three times 5 min with TNT buffer and two times 1 min with twice-distilled water.

(4) Silver enhancement was performed according to Danscher's method for 15 min at RT in the dark. The slides were then washed three times 3 min in TNT buffer and dried in the air.

Results observed with reflection microscopy

The oligo-probe hybridization showed a specific silver staining, especially for the 1q12 probe.

EXAMPLE 14

Preparation of gold particles coated with disulfide-containing-spacer-bound biotin (Biotin^{SS}) for purification purposes

Biotin^{SS}: NHS-O-C(=O)-$(CH_2)_2$-S-S-$(CH_2)_2$-NH-C(=O)-$(CH_2)_4$-Biotin

NHS = N-succinimidyl

Preparation

(1) To 11.0 ml of DMSO under stirring were subsequently added: 200 µl of a 0.10% MPS solution in ethanol, and 400 µl of a 10 mM $HAuCl_4$ solution in a 3:1 (v/v) DMSO/ethanol mixture. After stirring for 1 min, 400 µL of a 100 mM $NaBH_4$ solution in H_2O (twice distilled) was added (sol code Au(Dm,3.78)).

(2) 200 mM APMS-SATA and 100 mM APMS-Biotin^{ss} solutions in DMSO were prepared by stoichiometrically adding the reagents APMS, SATA and NHS-Biotin^{ss} and letting them react for one day at RT.

(3) After 15 min of stirring sol Au(Dm,3.78), 500 µL of triethylamine (TEA, 99%) was added and the sol was stirred for 30 min at RT. Then subsequently were added: 60 µL of the 200 mM APMS-SATA solution in DMSO and 20 µL of the 100 mM APMS-Biotin^{ss} solution in DMSO (SATA:Biotin^{ss}=6:1). This solution was stirred for about 30 min at RT. Then, purification was performed by gel filtration over a Sephadex LH-20 column, eluded with DMSO (sol code Au(Dm,3.78)-Biotin^{ss}-SATA).

EXAMPLE 15

Purification of immunoglobulin-gold-Biotin^{ss} conjugates by streptavidin-coated magnetic beads

Strategy

In order to purify immunoglobulin-gold conjugates, the gold particles are used that have been coated with both SATA and biotin^{ss}. The SATA groups are used to couple the gold particles to Goat IgG anti dinitrophenol (GαDNP), using the SMCC method (described in Example 8). The disulfide-containing biotins on the gold particles serve to extract the IgG-gold conjugates from the solution. After free gold particles were removed by gel filtration (fraction 1), streptavidin-coated magnetic beads were added. Only immunoglobulin-gold conjugates can bind to the streptavidin beads. These beads were collected using a magnet and the supernatant, containing unlabeled immunoglobulins, was removed (fraction 2). Immunoglobulin-gold conjugates were separated from the beads by an incubation with DTT which cleaves the sulfhydryl bridge between the biotin and the (immunoglobulin bound) gold particle (fraction 3).

Method

(1) One ml of GαDNP (1 mg), labeled with biotinylated-SS-gold particles (GαDNP-Au-Biot^{SS}, fraction 1) and purified by Sephadex G-50 Fine (Pharmacia) in PBSE, was mixed with streptavidin-coated dynabeads M-280 (50 µl, Dynal), washed with PBSE. After a 15 min incubation at RT, the tube was placed in a magnet (Dynal) and the supernatant was removed (fraction 2). The dynabeads were washed 3 times with 1 ml PBSE. Then 0.2 ml DTT solution (50 mM DTT in 0.1 M sodium acetate/acetic acid pH4.5) was added and after a 30 min incubation at 37°C, the tube was placed in a magnet. The supernatant was removed and added to 0.8 ml 0.2 M sodium phosphate pH8.0 (fraction 3).

(2) One µl of each fraction was spotted onto 3 nitrocellulose filters (Schleier and Schuell, 0.45 µm). The filters were allowed to air-dry and were subsequently incubated in either alkaline phosphatase-conjugated Rabbit IgG anti goat IgG (Sigma, 1 µg/ml in TNB) or alkaline phosphatase-conjugated streptavidin (Vector, 2 µg/ml in TNB). After a 30 min incubation at RT, the two filters were washed 3 times 3 min in TNT, followed by two short rinses in bidest. The filters were then stained for 1 to 2 h at RT in a NBT/BCIP solution (Rockland). The third filter was stained with silver enhancer according to Danscher's method.

Results

In resumption, all collected fractions have been spotted onto nitrocellulose and stained either with streptavidin-alkaline phosphatase, Rabbit anti-goat IgG-alkaline phosphatase or by silver enhancement of the gold particles. Alkaline phosphatase has been stained with NBT/BCIP.

Fraction 1 could be stained by any method. This proves that fraction 1 contained immunoglobulins and that the labeling with the biotinated gold particles was, at least partly, successful.

Fraction 2 could only be stained with the anti-goat IgG antibody, thus consisted of free immunoglobulins.

Fraction 3 showed staining by the anti-goat IgG antibody and the silver enhancement but not with streptavidin-alkaline phosphatase. Thus, fraction 3 contained immunoglobulin-gold conjugates, free of biotin labels.

LIST OF ABBREVIATIONS

SATA: N-succinimidyl S-acetyl thioacetate
SIAB: N-succinimidyl (4-iodoacetyl) aminobenzoate
SMCC: succinimidyl 4-(N-maleimidomethyl) cyclohexane 1-carboxylate
MPS: γ-mercaptopropyl trimethoxysilane
APS: γ-aminopropyl triethoxysilane
APMS: γ-aminopropyl methyl diethoxysilane
TEA: triethyl amine
TPM: γ-trimethoxysilyl propyl methacrylate

CONJUGATION BUFFERS

5×NHS buffer: 250 mM (Na)phosphate pH8.0/500 mM NaCl/25 mM EDTA.
5×SATA buffer: 250 mM (Na)phosphate pH7.4/500 mM NaCl/25 mM EDTA.
5×MEI buffer: 250 mM (Na)phosphate pH6.8/500 mM NaCl/25 mM EDTA.

BUFFERS FOR FILTER TESTS

TNB buffer: 50 mM Tris-HCl pH 7.4/150 mM NaCl/0.5% (w/v) blocking reagent (Boehringer).
TNT buffer: 50 mM Tris-HCl pH 7.4/150 mM NaCl/0.05% (v/v) Tween 20 (Sigma).
DAB/Ni solution: 0.05% (w/v) diaminobenzedine (DAB, Sigma)/ 50 mM Tris-HCl pH 7.4/10 mM imidazole (Sigma)/0.2% (w/v) $NiCl_2$/0.03% (v/v) H_2O_2.

BUFFERS USED IN HYBRIDIZATION EXPERIMENT (Example 13)

2×SSPE: 0.3 M NaCl/20 mM Na phosphate pH 7.2/4 mM EDTA.
1×SSTE buffer: 0.15 M NaCl/50 mM Tris-HCl pH 7.5/5 mM EDTA.
1×SSC buffer: 0.15 M NaCl/15 mM sodium citrate/pH 7.5.
Ureum hybmix: 3.5 M ureum/10% dextrane sulfate/5×Denhardts 10 μg/ml hsDNA/50 mM Tris-HCl pH 7.5/150 mM NaCl/5 mM EDTA.

CLAIMS

1. A metal particle comprising a metal core and a silane shell, wherein said core comprises a metal selected from the group consisting of gold, silver, platinum, palladium, rhodium, ruthenium, osmium, iridium and combinations thereof, and said shell comprises a mercaptosilane residue bound to said metal core.

2. The metal particle of claim 1, wherein said mercapto-silane residue is derived from a mercaptosilane compound of the formula $HS-(CH_2)_m-Si(OR^1)_nR^2_{3-n}$, wherein m and n both are integers, $m \geq 0$, $0 \leq n \leq 3$, each R^1 independent from any further R^1's is a member of the group consisting of hydrogen, alkyl and trialkylsilyl, and each R^2 independent from any further R^2's is a member of the group consisting of alkyl, haloalkyl, phenyl and halogen.

3. The metal particle of claim 2, wherein

--- $0 \leq m \leq 18$, preferably $1 \leq m \leq 6$, most preferably $m = 3$,

--- $0 \leq n \leq 3$, preferably $1 \leq n \leq 3$,

--- R^1 is hydrogen, C_1-C_6 alkyl, preferably C_1-C_4 alkyl, most preferably methyl or ethyl, or C_1-C_4 trialkylsilyl, most preferably trimethylsilyl, and

--- R^2 is C_1-C_{18} alkyl, preferably C_1-C_6 alkyl, halo(C_1-C_{18})-alkyl, preferably halo(C_1-C_6)alkyl, phenyl, or halogen, wherein halo(gen) is selected from the group consisting of F, Cl, Br and I.

4. The metal particle of any one of claims 1 to 3, which has a size of 5 nm or lower, preferably a size in the range of from about 0.8 nm to about 1.5 nm.

5. The metal particle of any one of claims 1 to 4, wherein mercaptosilane residues in said shell are cross-linked with each other.

6. The metal particle of any one of claims 1 to 5, wherein said silane shell is surrounded by a further shell, said

further shell comprising a silane residue, a silicate residue, a titanate residue, a zirconate residue, an aluminate residue, or a borate residue.

7. The metal particle of claim 6, wherein said further shell comprises a silane residue derived from an organosilane compound of the formula $X-(CH_2)_m-Si(OR^1)_nR^2_{3-n}$, wherein m and n both are integers, $m \geq 0$, $0 \leq n \leq 3$, each R^1 independent from any further R^1's is a member of the group consisting of hydrogen, alkyl and trialkylsilyl, each R^2 independent from any further R^2's is a member of the group consisting of alkyl, haloalkyl, fenyl and halogen, and X is a functional group.

8. The metal particle of claim 7, wherein

--- $0 \leq m \leq 18$, preferably $1 \leq m \leq 6$, most preferably $m = 3$,

--- $0 \leq n \leq 3$, preferably $1 \leq n \leq 3$,

--- R^1 is hydrogen, C_1-C_6 alkyl, preferably C_1-C_4 alkyl, most preferably methyl or ethyl, or C_1-C_4 trialkylsilyl, most preferably trimethylsilyl,

--- R^2 is C_1-C_{18} alkyl, preferably C_1-C_6 alkyl, halo(C_1-C_{18})-alkyl, preferably halo(C_1-C_6)alkyl, phenyl, or halogen, wherein halo(gen) is selected from the group consisting of F, Cl, Br and I, and

--- X is selected from the group consisting of amino, thiol, carboxyl, aldehyde, dimethyl acetal, diethyl acetal, epoxy, cyano, isocyanate, acyl azide, anhydride, diazonium salt, sulfonate, hydroxyphenyl, aminophenoxy, halogen, acetate, acrylate, methacrylate and vinyl.

9. The metal particle of any one of claims 6 to 8, wherein residues in said further shell are cross-linked with each other.

10. The metal particle of any one of claims 1 to 9, which is a colloidal particle.

11. The metal particle of any one of claims 1 to 10, which carries a foreign molecule covalently attached to a silane residue, preferably to a silane residue in said further shell.

12. The metal particle of claim 11, wherein said foreign molecule is selected from the group consisting of antibodies (immunoglobulines), antigens, haptens, biotin, avidin, streptavidin, protein A, proteins, enzymes, lectins, hormones, nucleic acids (DNA, RNA, oligonucleotides), fluorescent compounds and dyes.

13. A material or a device comprising a matrix or support having embedded therein or carrying a particle as defined in any one of claims 1 to 12, wherein said matrix or support is preferably selected from the group consisting of polymers, porous glass, colored glass, porous TiO_2, zeolites, silica, alumina, intercalated clay compositions, active carbon, graphite, ion-exchange resins, and semi-conductors such as p-GaAs, TiO_2, ZnO, CdS and Cd_3P_2.

14. A method of making a metal particle comprising a metal core and a silane shell, wherein said core comprises a metal selected from the group consisting of gold, silver, platinum, palladium, rhodium, ruthenium, osmium, iridium and combinations thereof, and said shell comprises a mercapto-silane residue bound to said metal core, comprising treating a solution of a compound of the metal with a reducing agent in the presence of a mercaptosilane compound.

15. The method of claim 14, wherein the metal compound is selected from the group consisting of $HAuCl_4$, H_2PtCl_6, $PdCl_2$, $AgNO_3$, and similar Au, Pt, Pd and Ag salts.

16. The method of claim 14, wherein the metal compound is dissolved in a polar solvent, preferably a polar solvent having a dielectric constant of 15 or higher, most preferably methanol, ethanol, dimethylsulfoxide, or dimethylformamide.

17. The method of claim 14, wherein the reaction is carried out in the presence of water, preferably from about 1 to about 10% by weight.

18. The method of claim 14, wherein said reducing agent is a borohydride, such as sodium borohydride.

19. The method of claim 14, wherein said mercaptosilane compound has the formula $HS-(CH_2)_m-Si(OR^1)_nR^2_{3-n}$, wherein m and

n both are integers, each R^1 is independent from any further R^1's and each R^2 is independent from any further R^2's,

--- $0 \leq m \leq 18$, preferably $1 \leq m \leq 6$, most preferably $m = 3$,

--- $0 \leq n \leq 3$, preferably $1 \leq n \leq 3$,

--- R^1 is hydrogen, C_1-C_6 alkyl, preferably C_1-C_4 alkyl, most preferably methyl or ethyl, or C_1-C_4 trialkylsilyl, most preferably trimethylsilyl, and

--- R^2 is C_1-C_{18} alkyl, preferably C_1-C_6 alkyl, halo(C_1-C_{18})-alkyl, preferably halo(C_1-C_6)alkyl, phenyl, or halogen, wherein halo(gen) is selected from the group consisting of F, Cl, Br and I.

20. The method of claim 14, wherein the reducing agent and the metal compound are used in a molar ratio of 7 or higher.

21. The method of claim 14, wherein the metal compound and the mercaptosilane compound are used in a molar ratio of 6 or lower.

22. The method of any one of claims 14 to 21, wherein the metal particle comprising said silane shell is subjected to a further reaction, to surround said silane shell by a further shell, said further shell comprising a silane residue, a silicate residue, a titanate residue, a zirconate residue, an aluminate residue, or a borate residue.

23. The method of claim 22, wherein the metal particle comprising said silane shell is subjected to a silane cross-linking reaction with an organosilane compound of the formula $X-(CH_2)_m-Si(OR^1)_nR^2_{3-n}$, wherein m and n both are integers, each R^1 is independent from any further R^1's and each R^2 is independent from any further R^2's,

--- $0 \leq m \leq 18$, preferably $1 \leq m \leq 6$, most preferably $m = 3$,

--- $0 \leq n \leq 3$, preferably $1 \leq n \leq 3$,

--- R^1 is hydrogen, C_1-C_6 alkyl, preferably C_1-C_4 alkyl, most preferably methyl or ethyl, or C_1-C_4 trialkylsilyl, most preferably trimethylsilyl,

--- R^2 is C_1-C_{18} alkyl, preferably C_1-C_6 alkyl, halo(C_1-C_{18})-alkyl, preferably halo(C_1-C_6)alkyl, phenyl, or halogen, wherein halo(gen) is selected from the group consisting

of F, Cl, Br and I, and

--- X is selected from the group consisting of amino, thiol, carboxyl, aldehyde, dimethyl acetal, diethyl acetal, epoxy, cyano, isocyanate, acyl azide, anhydride, diazonium salt, sulfonate, hydroxyphenyl, aminophenoxy, halogen, acetate, acrylate, methacrylate and vinyl.

24. The method of claim 22 or 23, wherein the reaction is carried out in a polar solvent, preferably a polar solvent having a dielectric constant of 15 or higher, most preferably methanol, ethanol, DMSO or DMF, in the presence of water, preferably from about 1 to about 10% by wt.

25. The method of any one of claims 22 to 24, wherein the reaction is carried out in the presence of a catalyst for siloxan bond formation, such as an amine or ammonia.

26. The method of any one of claims 14 to 25, further comprising a reaction to covalently attach a foreign molecule to a silane residue.

27. The method of claim 26, wherein said foreign molecule is attached to a silane residue in said further shell.

28. The method of claim 27, wherein the silane residue in said further shell is derived from an organosilane compound of the formula $X-(CH_2)_m-Si(OR^1)_nR^2_{3-n}$, wherein m and n both are integers, each R^1 is independent from any further R^1's and each R^2 is independent from any further R^2's,

--- $0 \leq m \leq 18$, preferably $1 \leq m \leq 6$, most preferably $m = 3$,

--- $0 \leq n \leq 3$, preferably $1 \leq n \leq 3$,

--- R^1 is hydrogen, C_1-C_6 alkyl, preferably C_1-C_4 alkyl, most preferably methyl or ethyl, or C_1-C_4 trialkylsilyl, most preferably trimethylsilyl,

--- R^2 is C_1-C_{18} alkyl, preferably C_1-C_6 alkyl, halo(C_1-C_{18})-alkyl, preferably halo(C_1-C_6)alkyl, phenyl, or halogen, wherein halo(gen) is selected from the group consisting of F, Cl, Br and I, and

--- X is a functional group used for coupling the foreign molecule, and is selected from the group consisting of amino, thiol, carboxyl, aldehyde, dimethyl acetal,

diethyl acetal, epoxy, cyano, isocyanate, acyl azide, anhydride, diazonium salt, sulfonate, hydroxyphenyl, aminophenoxy, halogen, acetate, acrylate, methacrylate and vinyl.

29. The method of any one of claims 26 to 28, wherein said foreign molecule is selected from the group consisting of antibodies (immunoglobulines), antigens, haptens, biotin, avidin, streptavidin, protein A, proteins, enzymes, lectins, hormones, nucleic acids (DNA, RNA, oligonucleotides), fluorescent compounds and dyes.

30. The method of any one of claims 26 to 29, wherein said foreign molecule is attached to a silane residue in said further shell after formation of said further shell.

31. The method of any one of claims 26 to 29, wherein, to obtain a metal particle having the foreign molecule attached to a silane residue in said further shell, said foreign molecule is attached to the organosilane compound which is then used for making said further shell.

32. Use of a metal particle as defined in any one of claims 1 to 12 in nanoelectronic devices and methods, in (photo) catalytic materials and methods, in monoliths and coatings, as a label or labeled reagent in immunoassay kits and immunoassay methods, or as a label or labeled probe in nucleic acid detection kits and methods.

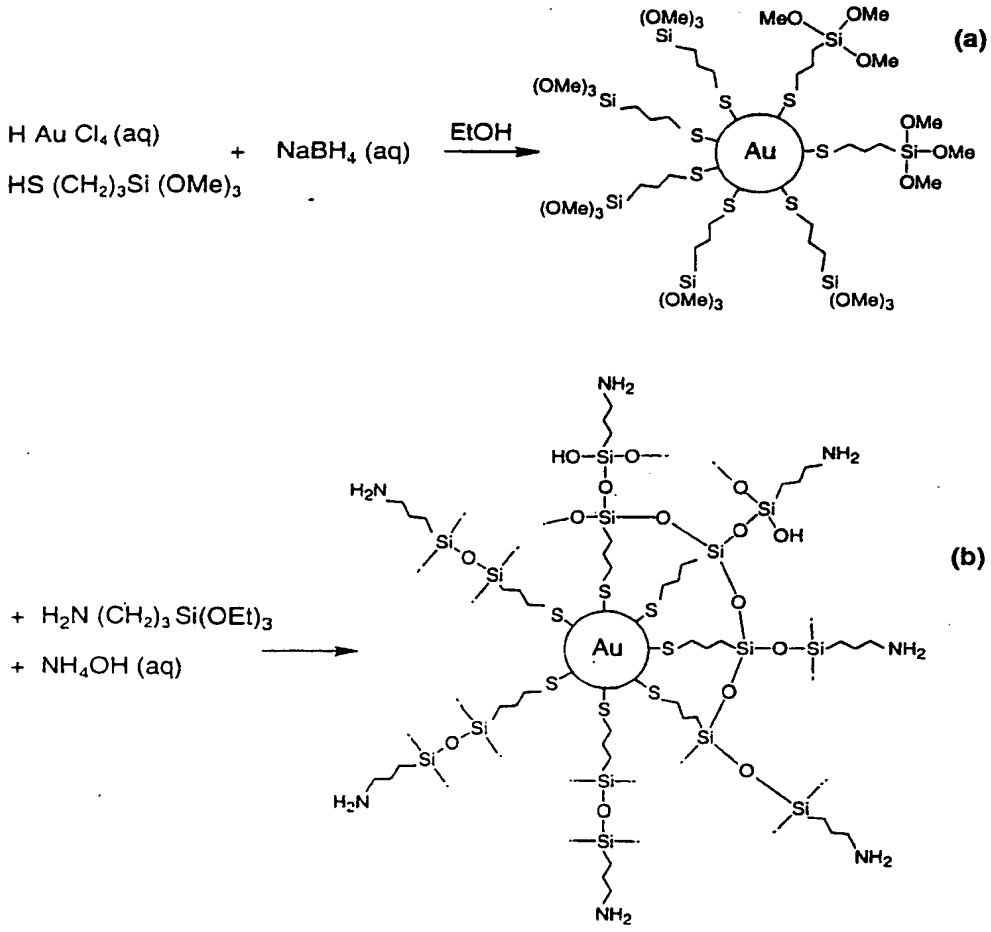
H Au Cl4 (aq)
HS (CH2)3Si (OMe)3
+
NaBH4 (aq)
EtOH
(a)
Au
+ H2N (CH2)3 Si(OEt)3
+ NH4OH (aq)
(b)
Au

FIGURE 1

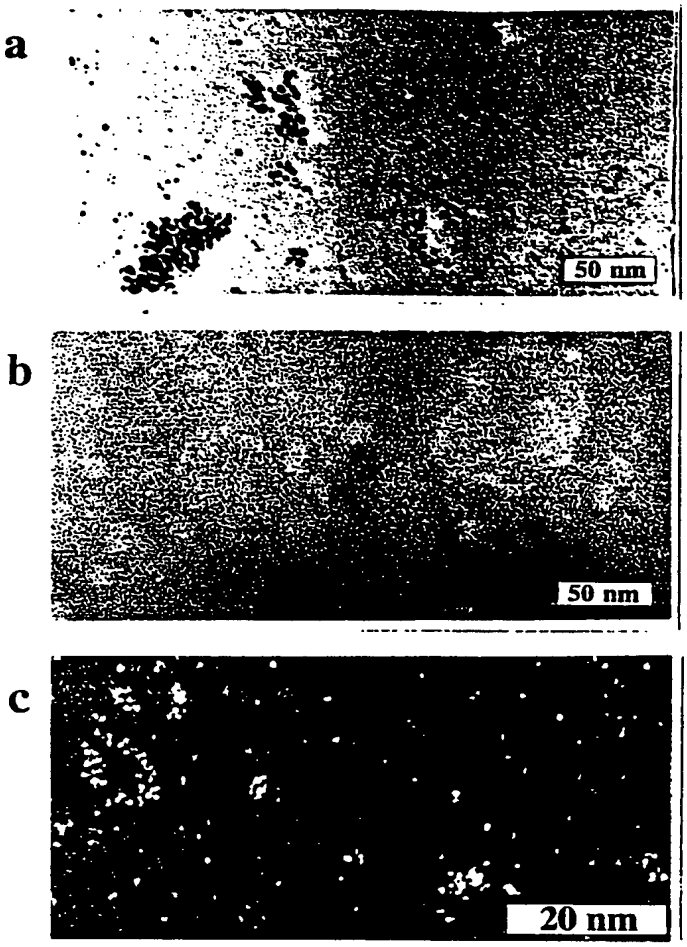

FIGURE 2

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/NL 97/00381

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N33/543 G01N33/58 B01J13/00

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N B01J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
T	BUINING, PAUL A. ET AL: "Preparation of Functional Silane-Stabilized Gold Colloids in the (Sub) nanometer Size Range" LANGMUIR (1997), 13(15), 3921-3926 CODEN: LANGD5;ISSN: 0743-7463, XP002056203 see the whole document ---	1-32
A	EP 0 489 465 A (AKZO NV) 10 June 1992 see the whole document ---	1
A	WO 96 18645 A (MERCK PATENT GMBH ;NAUMANN RENATE (DE); JONCZYK ALFRED (DE)) 20 June 1996 see the whole document ---	1
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

1

Date of the actual completion of the international search	Date of mailing of the international search report
19 February 1998	06/03/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Wells, A

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 97/00381

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category ³	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
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A	CHUMANOV, GEORGE ET AL: "Unusual Extinction Spectra of Nanometer-Sized Silver Particles Arranged in Two-Dimensional Arrays" J. PHYS. CHEM. (1996), 100(13), 5166-8 CODEN: JPCHAX;ISSN: 0022-3654, XP002056205 see the whole document ---	1
A	SARATHY VIJAYA K. ET AL: "A Novel Method of Preparing thiol-derivatised nanoparticles of gold, platinum and silver forming superstructures" CHEMICAL COMMUNICATIONS, 1997, pages 537-538, XP002056206 cited in the application see the whole document -----	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ional Application No
PCT/NL 97/00381

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